# ONTOGENY OF THE ANNULUS VENTRALIS.

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In crayfish of the genus *Cambarus* there is a sperm receptacle in the female which has been known as the *Annulus ventralis* and made use of as a specific character in systematic works.

Nothing has been known of its mode of development beyond the brief mention by Mary Steele [1] who figured the external views of the annulus in specimens of *C. gracilis*, 20, 22, 27.5, 30, 35, 36, 50 and 60 mm. in length. Of these the latter four were probably adults and the former four immature young. These figures show an increasing complexity of external sculpturing and an increase in relative longitudinal diameter; however, as they were made incidentally and without reference to the use or internal structure of the organ they are necessarily insufficient. While the annulus is a necessary reproductive organ in *Cambarus* it is also a new organ in the history of crayfish since it is found only in the most specialized form, *Cambarus*. It seemed, therefore, of interest to find out just how and when the organ develops in the ontogeny of *Cambarus*.

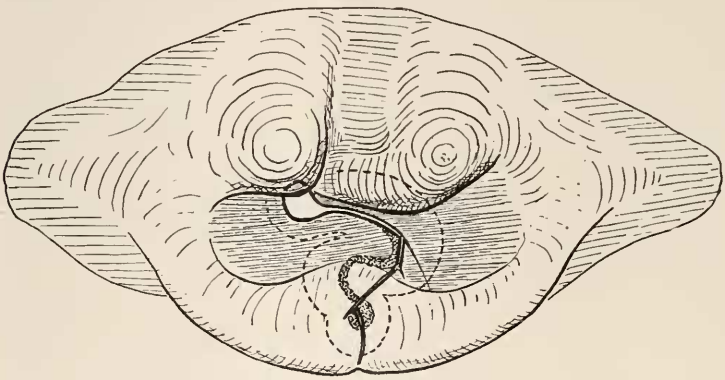

FIG. 1.

The following account of the origin and growth of the annulus refers almost exclusively to *Cambarus affinis* reared in the labo-

[1] *Univ. of Cin. Bulletin*, X., 1902.

ratory. In large adults, 100 mm. long, the annulus in this species has the appearance outlined in Fig. 1, which is enlarged about twelve diameters. It is a transversely elongated plate, part of the shell, with a central depressed area bounded behind by a cross ridge and in front by two high tubercles or tuberosities. Across the depressed area runs a zigzag line which is in reality a closed suture whence a slit leads inward to a curved tube represented by the thick shaded line. The suture and the curved tube both open out on one side into the depressed area by an orifice partly under one of the tuberosities. The walls of the tube are thick chitinous continuations of the shell, as indicated by the broken lines. Underlying this chitinous mass is the epidermis which forms it and which was found to be folded in as a bent groove. A comparative study of annuli in several species showed that while the external sculpturing is various, the presence of a curved epidermal groove is constant and that, morphologically, this sperm receptacle is a bent epidermal pocket lined by chitin and opening to the exterior by a more or less closed slit.

The position of the annulus, as seen in Fig. 2 which is enlarged one and a half diameters, is on the ventral surface of the thorax

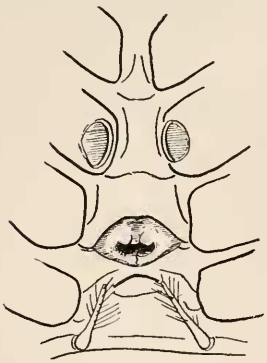

FIG. 2.

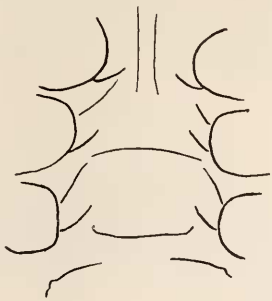

FIG. 3.

between the bases of the fourth pair of legs. The sternal surface behind it is elevated as a rounded knob that may be of importance in discharging the receptacle. Projecting forward towards this are the short pleopods of the first abdominal somite. Anteriorly, on the bases of the third pair of legs, are the large elliptical openings of the oviducts whence the eggs when laid pass back over the annulus.

In using the annulus as a sperm receptacle the male passes the sperm into the orifice and thence into the posterior part of the tube. The anterior part of the organ, the orifice itself and the following transverse part, which may be called the vestibule, is filled not with sperm but with a cement that protects the sperm from the water. Eventually the sperm issues out through the more posterior part of the suture at the right time to meet the eggs.

When the young *C. affinis* hatches from the egg there is no annulus present. The ventral surface (Fig. 3), multiplied fifty diameters, shows no specialization of the wide level area between the bases of the fourth legs. The sexes are not yet distinguishable and the first abdominal appendages are in all cases but very faintly indicated by slight elevations. Here under a higher power the epidermis was seen to be specialized as a group of nuclei, over which the cuticle was elevated as a slight protuberance in the region that is later to grow out as the first abdominal appendage.

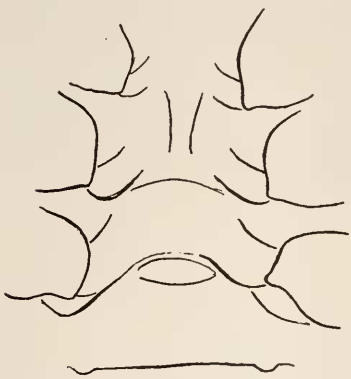

Fig. 4.

After this larva sheds its shell and passes into a second stage the sternal surface is larger (Fig. 4), and the first abdominal appendages are somewhat more protuberant. There is, however, no annulus and no external signs of sexual differences. Between the bases of the fifth legs there is now a transverse elevation of the sternum, which will become the prominent tubercle posterior to the annulus in the adult.

After a second moult the larva in the third stage has a much larger sternal area and for the first time sexual openings and the beginning of the annulus. In the female (Fig. 5), the sternum between the fourth legs is divided by a cross-line into an anterior part articulating with the legs, and a broad posterior region that is, however, markedly short. On the middle of this plate and at its posterior edge is a slight depression or groove destined to become the receptacle for sperm in the adult. In the male the same differentiation of a posterior sternal area is found, but the

plate lacks any groove and henceforth remains without any special development. The female is also recognizable by the appearance of the short, curved cuticular ridge on the base of the third leg, each side of the body, which is to be the median edge of the future orifice of the oviduct. From this superficial ridge a cylindrical epithelial tube, the oviduct, leads into the interior of the body. The first abdominal appendages, or pleopods, are blunt papillæ and apparently the same in both sexes, though sometimes in the male they seem more pointed and possibly longer.

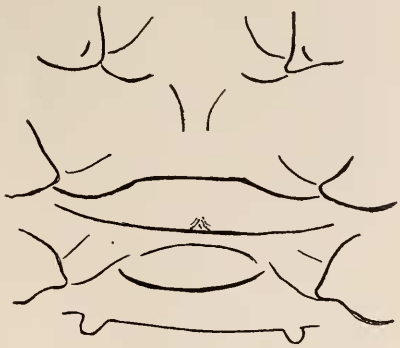

FIG. 5.

The annulus is thus a narrow transverse part of the sternum and since its posterior edge projects and overhangs somewhat it might be regarded as a sort of transverse fold. Its surface is entirely flat and simple as compared with the complex adult surface, Fig. 1, except for the slight median depression. Looking at the shell only, Fig. 6 enlarged two hundred diameters, this depression is a wide shallow groove indicated by converging wrinkles in the cuticle on each side the middle line of the animal and ex-

FIG. 6.

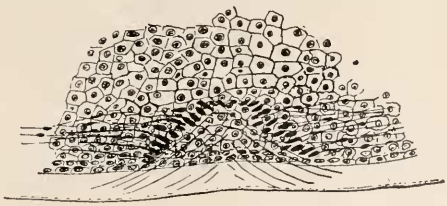

FIG. 7.

tending forward but a slight distance from the posterior edge of the annulus. The posterior edge of the annulus is rounded and protuberant and the groove to some extent extends over this posterior face of the annulus and cannot well be all seen at once from a ventral point of view. While this groove in the shell differs in different specimens it is in no way an artificial result of methods

of preservation but is based upon a special arrangement of the epidermis, which, Fig. 7 enlarged two hundred diameters, is a single layer of polygonal cells, close under the cuticle. This layer dips down under the groove and on the side of the groove, where seen in profile, the cells are elongated and continued as fine fibers that connect with connective tissue cells and with a membrane separating the epidermis from the large blood sinus just above it. In the figure these cells are represented in black as seen in optical section and also represented as seen in surface view upon another focus. Posteriorly the epidermis had shrunken away from the cuticle, in preparation, and left a wide artificial space. In an actual cross section of this region the groove is found to be just below the posterior part of the ganglion, Fig. 8, that supplies the fifth pair of legs, so that one might argue that the annulus belonged to the last thoracic somite while lying in the penultimate one. As seen in Fig. 8, which is enlarged two hundred

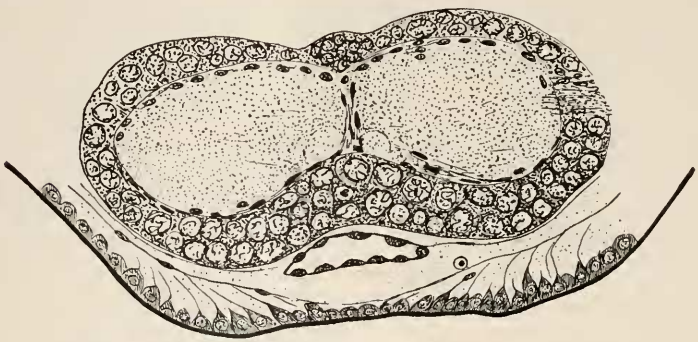

Fig. 8.

diameters, the epidermis cells fit together as polygons only at their outer ends next the shell while their inner ends taper off as fibers which diverge right and left of the central part of the groove. The epidermal cells are thus stretched out laterally and arranged on each side with reference to the superficial median groove that is to become the sperm receptacle.

Enclosing the epidermal cells is a coagulum of blood partly separated by a membrane from the blood space beneath the nerve cord. In this space lies the large median ventral artery that anterior to this section connects through the nerve cord with the

descending artery from the heart, between the fourth and fifth ventral thoracic ganglia.

Young crayfish in the above third stage are about 7–8 mm. long. In the fourth stage they are usually 11 mm. long. By this time the males and females differ noticeably in the lengths of the first pleopods. In the female, Fig. 9, they are still very small but larger than in the previous stage, this figure being enlarged

FIG. 9.

FIG. 10.

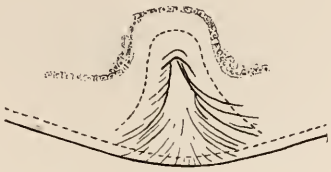

FIG. 11.

but thirteen, and Fig. 5, fifty diameters. In the male, Fig. 10, on the same scale, the pleopods are long, slender, but simple cylinders pointing toward one another. These two figures also show the increasing longitudinal diameter of the annulus in the female and its simple form in the male, as well as the reproductive openings upon the fifth legs in the male and the third legs in the female.

In these females 11 mm. long the median groove of the annulus is much more evident than at first, but is still a simple groove as shown in Fig. 11 enlarged 200 diameters. The walls of the folded in and thickening shell that bound the deep and narrow groove are indicated by the broken line. In this preparation the epidermis had shrunken far away from the shell and is indicated in optical section to show its invaginated state where it came under the cuticular groove. The groove seems to grow forward from the posterior face of the annulus and becomes more narrow and closed in anteriorly. At the anterior tip the groove was partly overhung by a cross fold, or tended to burrow under the surface as a short *cul de sac*.

In different females, however, the groove had different lengths and more or less of this covering-over of the front end. In a specimen 10 mm. long the groove was longer and more narrow than the one above figured. In others of 11 mm. the anterior

end was crossed by a sharp fold and the *cul de sac* was more pronounced. In some the posterior face of the annulus, which projected and overhung considerably, bore a curved transverse ridge strongly suggesting the posterior rim of the adult annulus, Fig. 1. In a large female of 13 mm. the groove was as in Fig. 11, though a little longer.

The single layer of epidermal cells under the shell was still visible as a series of polygons as in the third stage. While the annulus itself showed no setæ upon it in any stage the transverse ridge of the sternum behind it bore scattered over it some four or five sharp setæ in the third stage, about ten in the fourth and twenty in the fifth.

The fifth stage includes individuals 15–18 mm. long, and in them marked changes had taken place in the annulus, but before describing these we will refer to a few observations made upon the developing annulus in an early stage of another species, *C. Clarkii*, from New Orleans. Here also the young reared in the laboratory had no annulus in the first and second stages, which were about five and six millimeters long, and they also showed no external sexual differences. Young of this species were seen by Hagen,[1] when .3 inch long and still on the abdomen of the female; in which the females had no pleopods upon the first abdominal somite, but showed the openings of the oviducts, while the males had the first pleopods as little knobs longer than broad and turning inward. Later Faxon[2] stated that the young of this species from under the abdomen of the parent had the general form of the adult when 7 mm. long. Evidently both authors saw the third stage only.

Eggs hatched April 21 reached the third stage June 1, but no observations were made upon the annulus till they had passed into a later stage, June 19, and were 11 mm. long. In this condition the male and female were much like specimens of *C. affinis* of that length, but the first pleopods in the female were much smaller and in the male noticeably shorter than in *C. affinis*.

The groove on the annulus of the female now formed a deep, closed in cavity, Fig. 12, which is enlarged to the same extent as

[1] "Monograph N. A. Astacidæ," Harvard, 1870.

[2] "Revision of the Astacidæ," Harvard, 1885.

Fig. 11. In *C. Clarkii* the groove has closed, leaving a suture, represented by the black line, above an internal cavity, represented in white and this is bounded on the sides and bottom by the infolded cuticle, represented in dotted lines. At the posterior end the groove passes around the edge of the annulus and seems

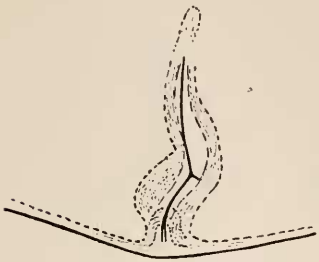

Fig. 12.

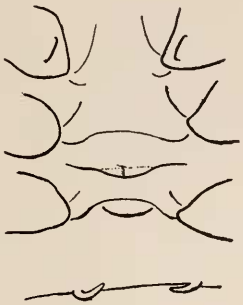

Fig. 13.

to be still open there. At the front end the groove pushed forward as a blind growth beneath the surface. Thus in *C. Clarkii* the receptacle though probably formed at the same stage and in the same way as in *C. affinis*, yet advances more rapidly, so that in the young 11 mm. long it is comparable, in its closed up condition, to *C. affinis* when 21 mm. long and in the sixth stage.

Returning to *C. affinis* in the fifth stage, 15–18 mm. long, the general appearance, Fig. 13, enlarged 13 diameters, shows an increase in size of the animal and a great growth of the first pleopods of the female which are now much longer than in the female of the fourth stage and nearly as long as the male pleopods of that stage, Fig. 10.

The receptacle itself is quite diverse in different individuals. In all there is added onto the median groove two elevations or folds which tend to cover over the anterior end of the groove. The groove itself is bent on one side more or less and the overhanging folds are more or less developed. In the specimen shown in Fig. 14 as enlarged 200 diameters the groove bends to one side and passes in under a marked flap or fold that grows over the tip of the groove. This lateral fold may be called the "hood" to distinguish it from the longer fold which passes along the opposite side of the groove and tends to overhang it.

This second fold is slightly developed in this case. In another specimen, Fig. 15, the groove bent very far to one side and the anterior part was concealed under the second fold. We will call this second fold the "transverse" fold, since it ultimately lies

FIG. 14.

FIG. 15.

more nearly across the median plane. Both folds are oblique, the "hood" and the "transverse" fold being about at right angles where the latter passes under the former. In Fig. 15 not only is much of the groove overhung by the transverse fold, but the entrance to the groove in under that fold is cut off, near the hood, by a short posterior fold that runs parallel to the transverse fold as it emerges from under the hood.

The cases of unusual bending of the groove to one side suggest the state of things found in the adults of *C. immunis* and *C. Bartoni* where the receptacle is more transversely placed than in *C. affinis*.

The specimen last figured was about to shed and the delicate new shell within the one here figured was more like that figured below for the next stage.

In another case the shell cast off by a larva going into the sixth stage had the groove but slightly bent, Fig. 16, and the

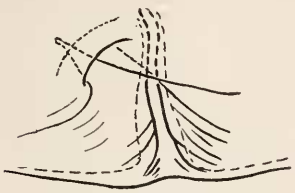

FIG. 16.

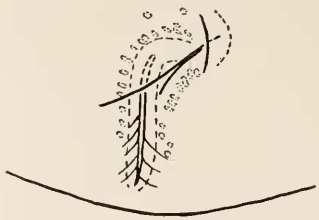

FIG. 17.

transverse fold was less oblique. As there was no fold opposing the transverse fold an object could have passed under its pos-

terior edge and thence to the anterior part of the groove. The receptacle was now being formed of three different parts: the original median groove, the two oblique folds. The former remains as the posterior part of the adult sperm tube and the latter help to make the orifice and the vestibule, or anterior part of the adult receptacle.

In all these cases the groove bends more or less to one side, and in only one case observed was the bending to the observer's right, which is the left of the animal. In this exceptional animal, Fig. 17, not only does the groove bend to the right, but the hood is on the right and the transverse fold and the posterior opposing fold run at right angles to their usual course. Comparison of Fig. 17 with Fig. 15, shows that they are, in the main, mirror-images of one another. Each reversed, as seen through the paper, would have the symmetry of the other.

This reversal of symmetry in the receptacles of some young is the first visible expression of the peculiar dimorphism of the annulus in the adults of this and other species. While many of the adults have the orifice upon the right side of the median plane others have it upon the left and in all respects these two forms of annulus are mirror-images of one another. Both forms are used as sperm receptacles. Those with the orifice upon the right, Fig. 1, are more common; in one lot of 41 females, 38 had these right-handed annuli.

The characteristic tuberosities of the adult grow out in later larval life and they also are right- and left-handed in the following way: while, usually, as in the above figure, the tuberosity upon the left of the animal sends a ridge under the right tuberosity, in left-handed adult annuli the behavior of the tuberosities is the reverse. In the production of the two symmetrical adult forms there is thus the harmonious development of the groove, folds and tuberosities at different periods and from several areas of the epidermis.

In July the larva may pass into a sixth stage 21 mm. long. On the ventral side, Fig. 18, enlarged thirteen diameters, there is a noticeable increase in the longitudinal diameter of the annulus and in the length of the first pleopods which are now longer than those of the male of stage four, Fig. 10, though closely applied

along the sternum and, apparently, of no use as yet. The annulus, however, has acquired a rather mature appearance. When enlarged fifty diameters, Fig. 19, it shows toward the center two gentle elevations to represent the future tuberosities and posteriorly a transverse curved rim like that of the adult. The whole plate is still narrow from before back but much less so than in previous stages. The suture of the receptacle is indicated by the zig-zag line and its lateral walls by the broken lines. The former represents: anteriorly on the left the former edge of the hood; posteriorly the closed up groove; and in its middle course the transverse fold.

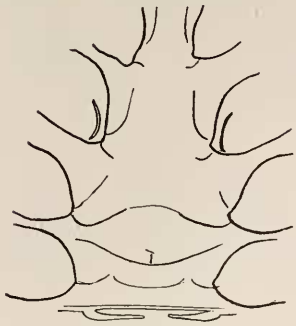

FIG. 18.

Enlarged 200 diameters, Figs. 20 and 21, the receptacle presents varieties of development in different individuals. The groove itself may be open posteriorly as in Fig. 20 or quite closed

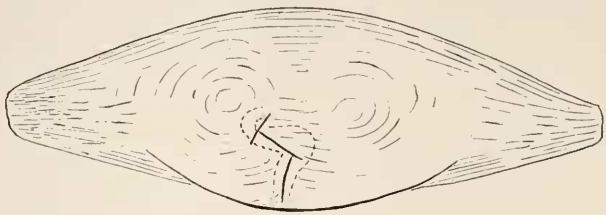

FIG. 19.

up as in Fig. 21. The anterior part of the groove bends far to one side to end under the hood at the right end of the transverse fold. This part of the groove may be still accessible by passing in under the transverse fold, Fig. 20, or it may be quite shut off by a posterior fold growing along against the posterior edge of the transverse fold as in Fig. 21. Fig. 20 is not much advanced beyond the preceding stage, Figs. 15 and 16, the open groove, the hood and the transverse fold are larger and the invaginated shell is more extensive, as indicated by the broken lines. But in other cases, Fig. 21, the deeply buried groove connects with the surface only by oblique planes that come to the surface as the three

successive parts of a zig-zag suture. The inner course of the groove is more sinuous than before in correspondence with the increased bending of the invaginated shell that forms its walls.

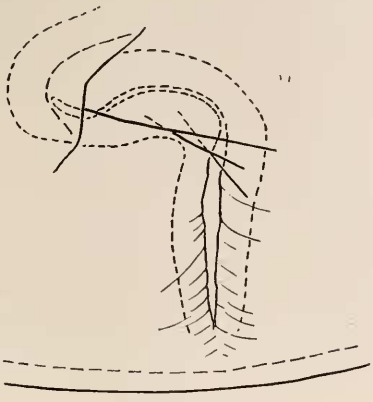

FIG. 20.

The chief features of the adult sperm pocket are thus present except that the anterior orifice seems more closed in some individuals.

Though in some cases it seemed as if the groove itself did not bend to one side to end under the hood, but rather that the transverse fold left a passage from the groove to the hood, the true state of the epidermal groove upon and in which the shell groove is formed was seen when the shell was pulled off. Then, in Fig. 22, enlarged 200 diameters, there is a deep, bent furrow with high sides formed of long epidermal cells, as somewhat diagrammatically represented in this camera lucida sketch of an optical section

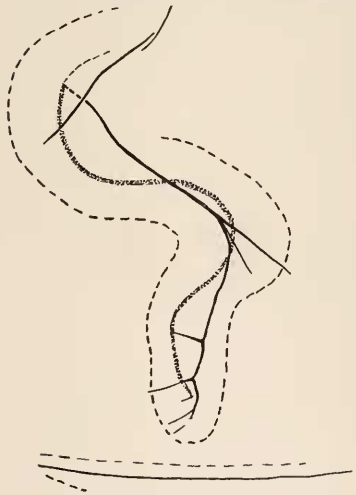

FIG. 21.

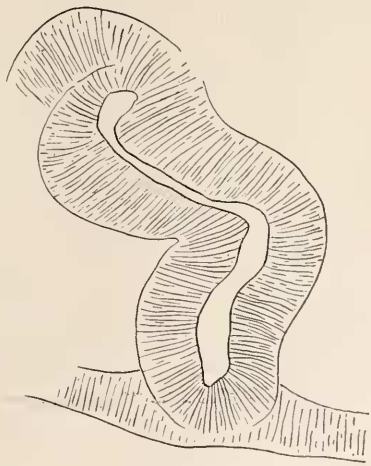

FIG. 22.

of the epidermal part of the receptacle. In this specimen the shell was easily removed since a new shell was in process of formation to

line the groove. This made the groove more distinct than otherwise is the case. The groove passes forward from the posterior edge of the annulus and then bends to one side much as does the bottom of the shell groove seen as a shaded line in Fig. 21. In each individual, however, the amount of bending seems to be different.

Such a bent groove is very like the simple bent epidermal groove of *C. Clarkii*, which is one of the less specialized species.

While previous experience had shown that larvæ after living eleven days in the above sixth stage might turn into a seventh stage 29 mm. long in the middle of July, no observations were made upon the above larvæ 21 mm. long till October 3, 1904, when they had turned into individuals 25–53 mm. long. Those 25–35 mm. agreed with one another in the development of the annulus and probably represented larvæ that from lack of enough food had remained in the seventh stage while the female, 53 mm. long, belonged to some later stage. In this laboratory it has been found that crayfish of that latter size have their sexual instincts developed in the fall and females 52 and 53 mm. long, being then supplied with sperm by males of the same or even smaller sizes, laid good eggs the following spring when not quite one year old. Thus the above female was probably mature in instincts and in external sexual organs when examined.

FIG. 23.

First, however, taking up the specimens 25–35 mm. long which were all essentially alike except in size, one 35 mm. long enlarged 13 diameters, Fig. 23, presents a noticeable growth of the first pleopods which are now turned forward and sparsely set with hairs as in the adult, and also much increase in the relative importance of the curved ridge bounding the mouth of the oviduct opening on the third leg. As an opaque object the annulus now

showed very definite tuberosities separate from the hood and the transverse fold and the tuberosity upon one side of the animal extended over the median line, in carrying out the asymmetry of the adult. The general proportions of the various folds and thickenings of the shell now approximated the adult condition.

The receptacle had not materially changed from the previous stage, Fig. 21, but its invaginated shell walls were greatly thickened and laminated, Fig. 24, enlarged like Fig. 21. It would appear that with the dropping of the bottom of the invaginated

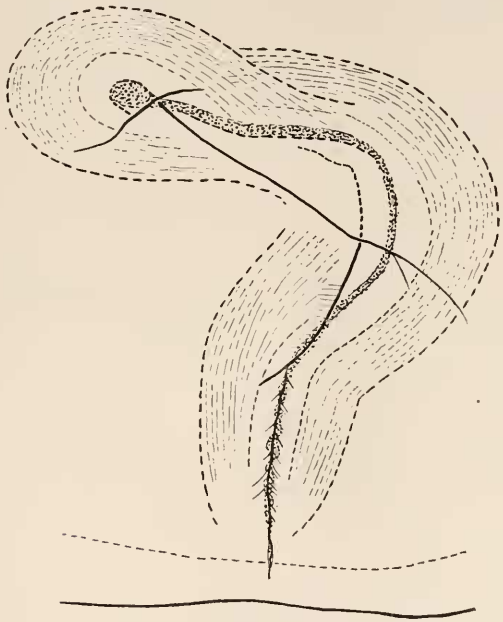

FIG. 24.

groove away from the surface its sides have closed in to form a narrow crevice which comes to the surface as a suture line. Then by the bending of the bottom of the invagination more than its surface suture line these crevices are made into curved oblique planes. The hood and the transverse fold and the posterior opposing fold are intimately associated with the bending of the original groove to one side and then the sinking of that lateral bend away from the surface and diagonally forward.

The last young to be considered, the larva, 53 mm. in length

and sexually mature, has an annulus very much like that of the full-grown adults, but it is still very small, as is seen on comparing Fig. 25 with Fig. 1, both enlarged 12 diameters. Still the tuberosities do not yet overhang the transverse depression enough to conceal the hood and the transverse fold, nor are the transverse depression and the posterior rim as well developed as later. The receptacle itself is much like that in Fig. 24, but its orifice is more perfect, though not yet as patent as in the large specimens, Fig. 1. This young annulus still lacks the posterior enlargement of the sperm tube, indicated in Fig. 1, and the complexity of bending of the tube is less, nevertheless it functions as a sperm receptacle.

FIG. 25.

## SUMMARY.

The specialized sternal plate of the shell of *Cambarus*, which in the adult female bears the sperm-receptacle, is first differentiated in the third larval stage, in *C. affinis*.

The epidermis under this plate grows inward and outward in special areas to form the receptacle. First there is formed an open median groove that then bends to one side, sinks away from the surface and becomes closed. In *C. Clarkii* also one stage shows a like development.

Next definite folds overgrow the anterior end of the groove as it sinks from the surface.

Later elevations and depressions complete the external sculpturing of the annulus.

The anterior part of the groove, with the accompanying folds, form the orifice and vestibule of the sperm receptacle, while the posterior part of the groove forms that part of the receptacle in which the sperm is stored.

A functionally complete annulus is made within five months, but subsequently it becomes more complex. Comparative study of the adults of several species has shown that in *Cambarus* the essential part of the annulus, as a sperm receptacle, is a curved pocket; the above facts indicate that in *Cambarus* in general this pocket arises as an open epidermal groove.

The right or left handed symmetry of different adult annuli is first visible in the fifth larval stage when the median groove has bent to the right or to the left and the accompanying folds have a reversed position in the two cases. Later other outgrowths may also harmonize with the groove and folds to complete the two adult forms; mirror-images of one another.

While the sperm receptacle is a necessary organ in *Cambarus* it is phylogenetically a new one since *Cambarus* is the most specialized genus of crayfish and other genera have no receptacle. In *C. affinis* this new organ is not seen till the individual has reached a third stage after leaving the egg.

Besides beginning late in ontogeny this new sperm receptacle is variable in all its stages of growth. In its early and simple state in the larva it resembles the adult receptacle of a less specialized species, *C. Clarkii*.

In some of its variations of lateral bendings in early larval stages it suggests adult conditions in other species.

BALTIMORE,
December 12, 1905.